

Fig. S1: Sagittal cervical spinal cord MRI of patient 14711. T1-weighted image after gadolinium injection; white arrows indicate contrast-enhancing spinal cord lesions at levels C3/4, C4, and C5/6.

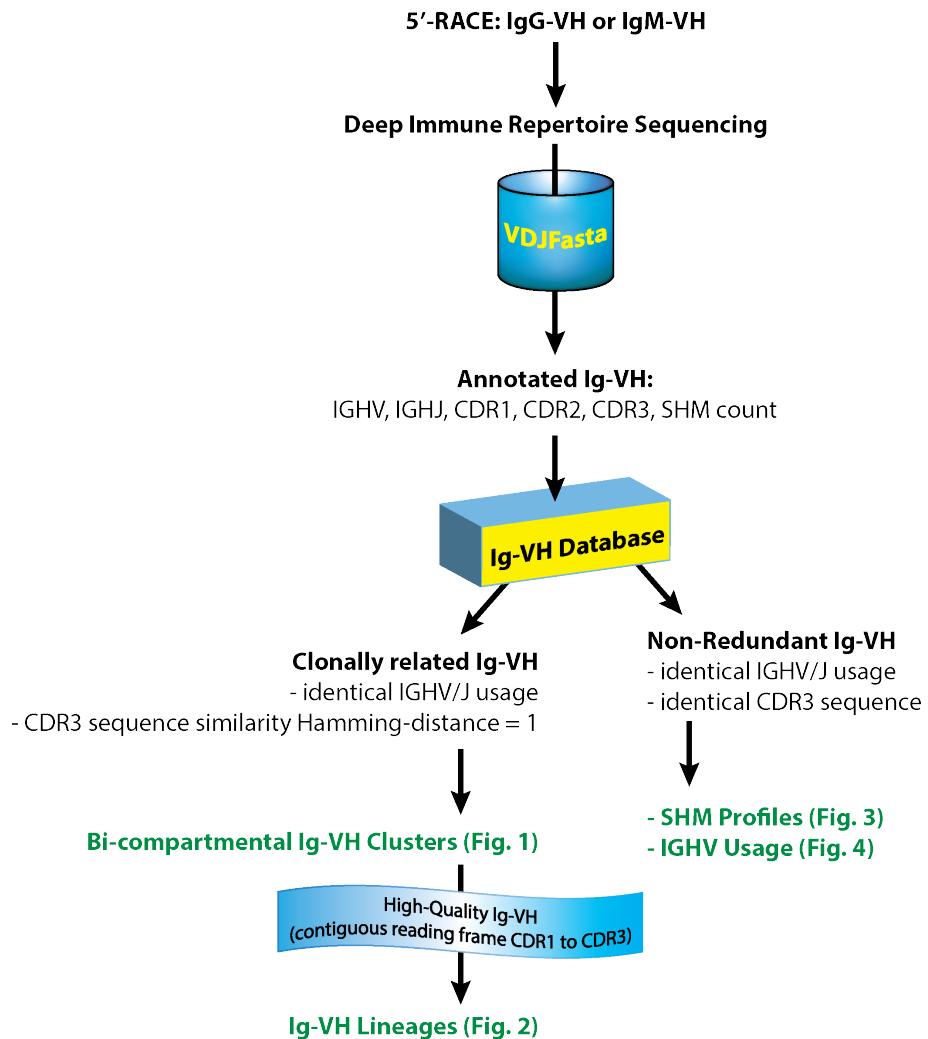


Fig. S2: Bioinformatics pipeline for Ig-VH analyses. Following 5'-RACE of CSF or sorted PB B cell subsets, IgM-VH and IgG-VH transcriptomes were sequenced by DIRS. Raw sequencing reads were analyzed by VDJFasta (reference 9 in main text) to determine germline IGHV/J, CDR, and SHM count for each sequence; SHM count was not influenced by indel sequencing errors. Ig-VH sequences were annotated with the VDJFasta generated information and entered into a database. From this Ig-VH database, clonally related Ig-VH were clustered based on IGHV/J usage and identical or highly similar H-CDR3 aminoacid sequence (Hamming distance=1). Ig-VH clusters including sequences from CSF and PB B cell subsets were defined as “bi-compartmental” Ig-VH clusters and identified PB B cell subsets that are connected to intrathecal B cell repertoires. To further analyze Ig-VH included in bi-compartmental clusters, we generated Ig-VH lineages using IgTree. For lineage generation only Ig-VH sequences with contiguous reading frames ranging at least from CDR1 to CDR3, i.e. sequences in which VDJFasta properly identified all CDRs connected by in-frame framework (FR) 2 and FR3. Thus, Ig-VH sequences including indels are not considered for lineage calculations. Representative lineages are shown in Figure 2; singleton sequences in leaves were treated as potential sequencing errors and combined triangle-shaped nodes; lineage displays were generated using Cytoscape 2.8 (reference 52 in main text). For calculations of IGHV usage and SHM profiles, we generated “non-redundant” datasets, i.e. datasets in which sequences with identical H-CDR3, IGHV and IGHJ are counted only once.

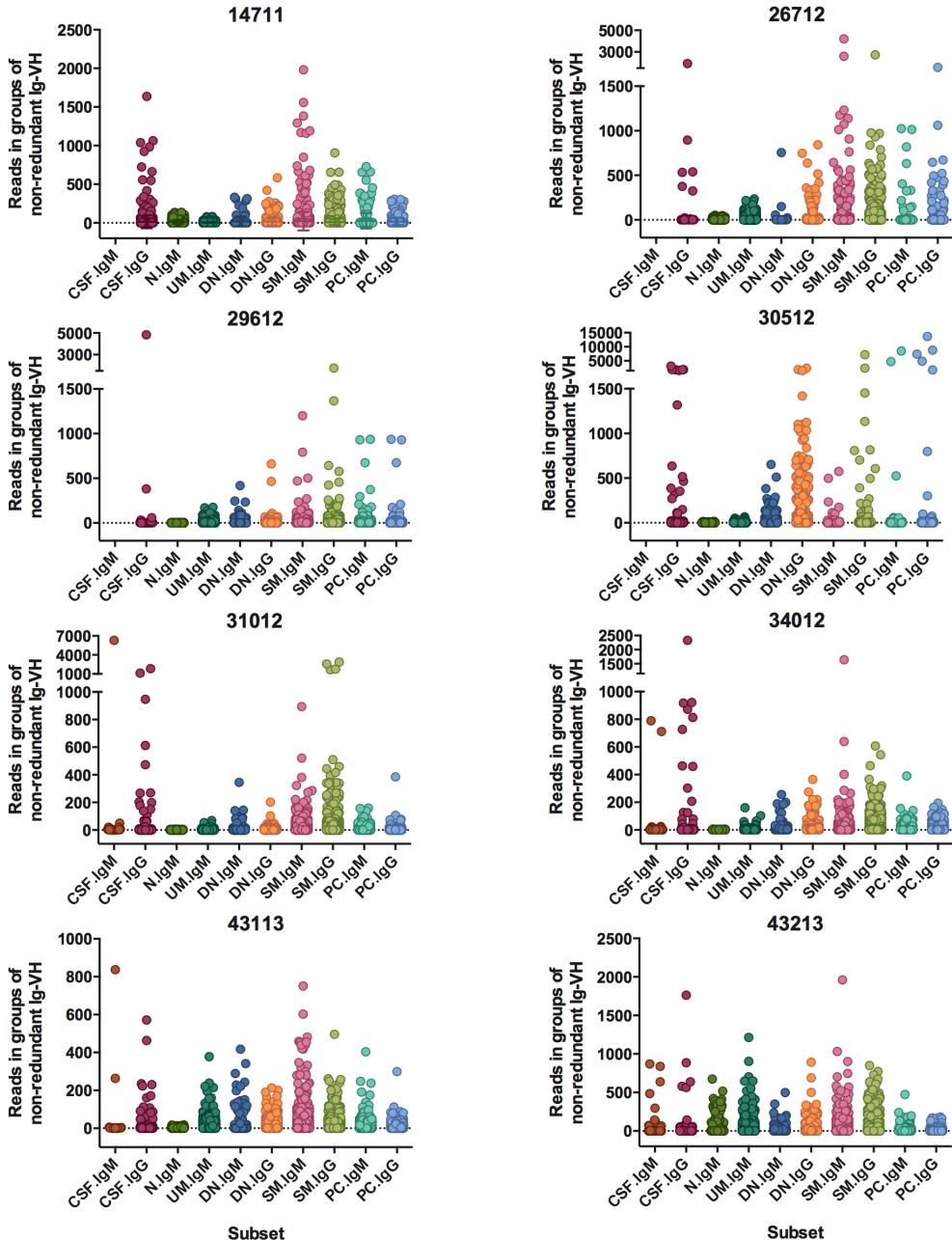


Fig. S3: Numbers of Ig-VH in CSF and B cell subsets approximate expected levels of antigen-stimulation. Shown are number of reads in each non-redundant Ig-VH sequence to provide an approximate measure of ‘clonal expansion’. Within each patient comparisons between subsets were made using a Kruskal-Wallis test (ANOVA with Dunn’s multiple comparisons); results and levels of statistical significance are shown in Table S7. Most notably, naïve B cells (N.IgM) and UM B cells (UM.IgM) commonly yielded significantly fewer numbers of reads in each non-redundant Ig-VH sequence, suggesting smaller groups of clonally related Ig-VH approximating absence of clonal expansion in these B cell subsets. Conversely, in CSF and Ig class-switched B cells clonal expansion is a common observation.

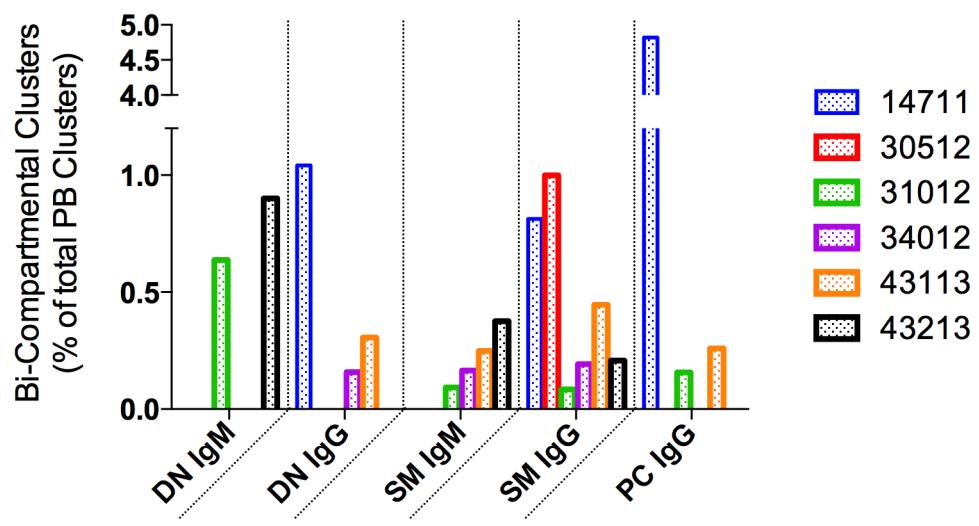


Fig. S4: Bi-compartmental clusters in proportion to total PB B cell clusters of the indicated PB B cell subset. Only subsets with connections to CSF immune repertoires are shown.

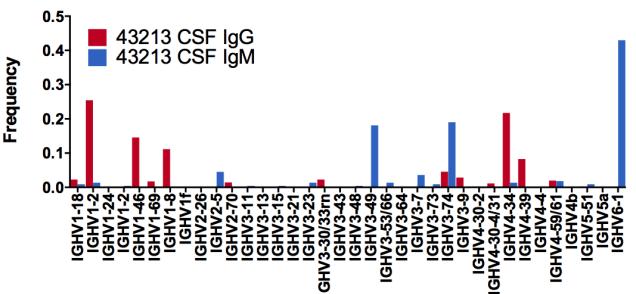
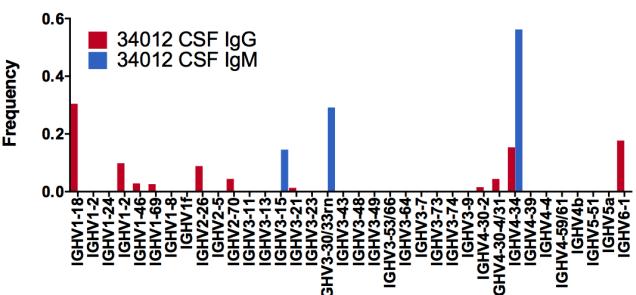
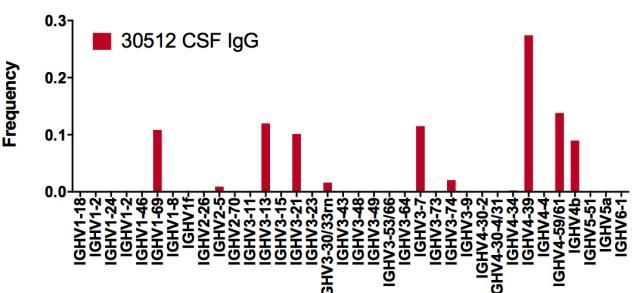
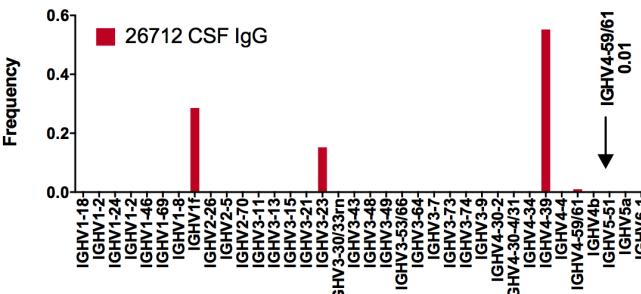
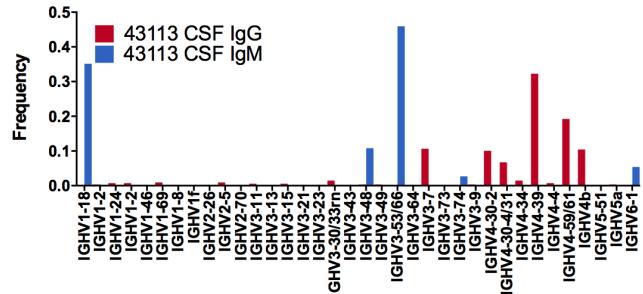
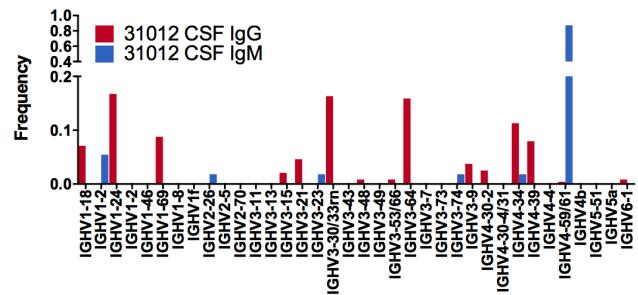
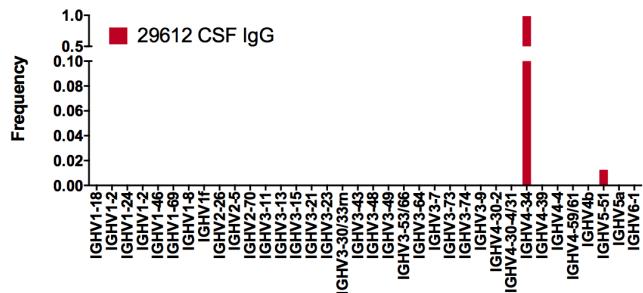
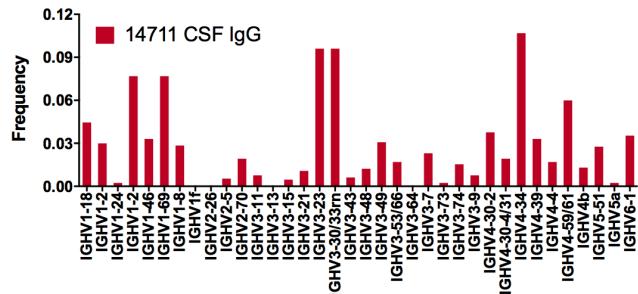


Fig. S6: IGHV usage profiles of CSF Ig-VH.

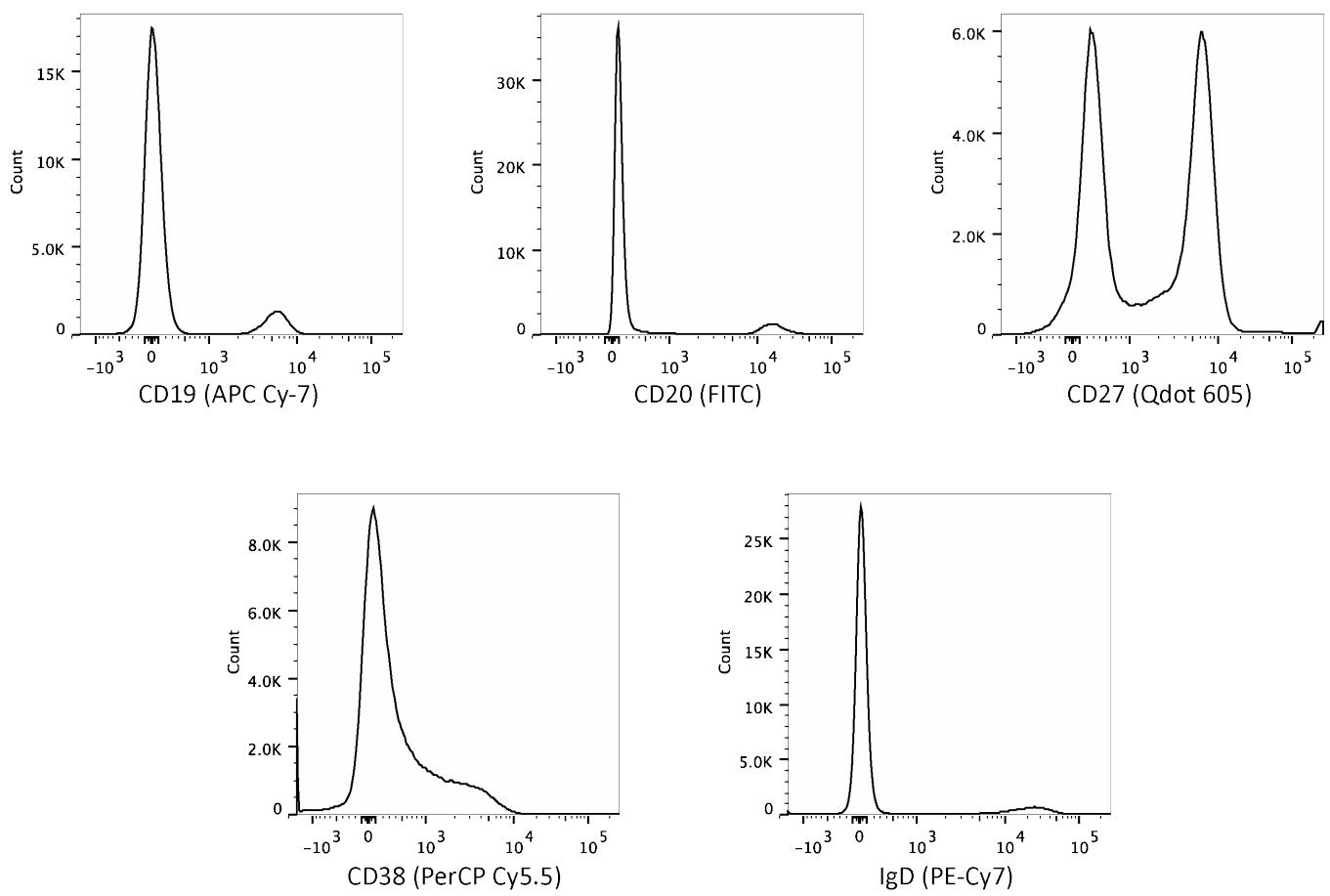


Fig. S7. Gating controls (single color histograms) for CD19, CD20, CD27, CD38, and IgD

Pat ID	Subset	%	N sorted	Total Reads	IgM-VH	IgG-VH	Non-redundant IgM-VH	Non-redundant IgG-VH
14711	CSF			23,323	20650		1290	
26712	CSF			7,793	4859		104	
29612	CSF			104,995	5638		79	
30512	CSF			34,521	18044		432	
31012	CSF			20,793	6604	7296	55	238
34012	CSF			23,999	6837	14121	144	383
43113	CSF			7,839	1151	4749	36	511
43213	CSF			13,687	3363	6099	219	345
14711	DN	4	32,458	14,303	2216	7372	109	401
26712	DN	2	10,522	10,336	1037	7577	37	283
29612	DN	7	253,478	17,199	2951	6209	201	638
30512	DN	2	57,253	104,553	10656	58312	668	2247
31012	DN	2	71,761	11,515	2459	5883	285	2095
34012	DN	3	19,934	15,587	2796	9977	351	1210
43113	DN	2	26,783	22,673	4087	12534	219	888
43213	DN	3	33,404	20,999	6564	11710	385	693
14711	N	72	201,268	21,233	18619		1385	
26712	N	67	99,987	17,819	15773		2445	
29612	N	59	200,457	7,245	6171		4918	
30512	N	63	200,000	23,723	19027		10663	
31012	N	9	150,740	10,465	8497		6872	
34012	N	76	418,458	18,624	13177		11417	
43113	N	62	324,564	16,590	14580		5337	
43213	N	77	125,519	17,110	15243		726	
14711	PC	0.2	695	17,593	7959	8044	289	425
26712	PC	0.6	952	19,465	5648	12498	179	452
29612	PC	1.8	23,010	20,677	6741	11649	1130	2280
30512	PC	0.2	1,715	57,277	14729	39704	165	297
31012	PC	2.1	10,885	14,177	6364	5733	698	1067
34012	PC	0.3	731	15,364	4504	9619	368	729
43113	PC	0.3	14,536	27,960	11106	14672	838	1725
43213	PC	1.4	13,016	26,161	10750	13473	1382	1578
14711	SM	11	125,537	80,499	32126	37838	1328	3187
26712	SM	12	89,280	79,688	28698	41347	811	1757
29612	SM	19	145,101	107,164	29476	60034	3250	9157
30512	SM	11	446,097	50,008	2082	19235	93	481
31012	SM	37	241,466	131,064	35752	80821	4405	12399
34012	SM	8	67,618	62,805	13857	40305	1299	5680
43113	SM	21	312,511	100,329	29031	47377	1577	4707
43213	SM	10	27,208	97,623	38220	51092	1983	2569
14711	UM	6	171,372	79,971	38537		6449	
26712	UM	9	70,171	43,705	20857		1468	
29612	UM	8	63,437	75,660	30377		3937	
30512	UM	20	189,904	180,910	37500		13538	
31012	UM	47	248,426	63,986	22527		12868	
34012	UM	7	75,455	47,908	17477		8143	
43113	UM	8	120,286	101,218	53706		5940	
43213	UM	4	14,911	103,109	43864		2906	

Table S1: Sorted cells and sequence counts. Shown are per patient B cell subsets in peripheral blood and numbers of sorted cells (N sorted) per subset. Percentages shown are relative to total B cell numbers. Total reads: number of total sequences resulting from the sequencing run. IgM-VH/IgG-VH: numbers of usable IgM- and/or IgG-VH sequences. Non-redundant IgM-VH/IgG-VH: numbers of non-redundant clusters IgM-VH or IgG-VH. N, naïve B cells (CD19+CD27-IgD+); UM, unswitched memory B cells (CD19+CD27+IgD+); SM, switched memory B cells (CD19+CD27+IgD-); DN, double negative B cells (CD19+CD27-IgD-); PC, plasma cells and plasmablasts (CD19+CD27^{hi}CD38^{hi}).

Pat	IGHV	H-CDR3	IGHJ	IgG (% reads)			IgM (% reads)		
				CSF	SM	PC	DN	CSF	SM
14711	1-18	CARDSRGFADIW	5	5.643	0.132	0.631			
	1-24	CATGGISSLDDWNYFYGLDLW	6	0.156		0.240			
	1-3	CAGPVWAGSPNWKPWFGVDW	6	8.867	0.093				
	1-8	CARVVRYKITWYFDPW	5	0.146		1.401			
	3-11	CARDRFGVFDFW	4	0.080	0.003				
	3-11	CARDLGRTRSIATVRGLLYDAFDIW	3	0.045	0.090				
	3-23	CAKDAGWYLYYYFDLW	2	2.274		0.776			
	3-30/33rn	CARRPEGYAMDVW	6	0.015	0.036				
	3-30/33rn	CAKDLVSDHYYYYGMDVW	6	0.271	0.036				
	4-34	CARGPKNKRFPMAPAEFFDYW	4	0.075	0.019				
	4-34	CARLGSSWWLLTALRSTKGQYYGMDV	6	1.054	0.096				
	4-4	CARGGFSITWGGFDW	3	0.181	0.003				
	4-59/61	CAKYDFWSGFDPW	5	0.201	0.063				
	4-b	CARSYGDYVDPFFDYW	4	0.321	1.888				
	6-1	CARSGKPTGGGVLAWGPKKFVSSLYFDS	4	5.754		0.101			
30512	2-5	CAHSKMATMGGELVFDYW	4	0.416	41.80		31012	30512	31012
31012	1-24	CATQSGMITASPLDYW	4	28.50	0.148				
	1-24	CATGRKSGVVGAYFDYW	4	0.502	0.063				
	3-21	CATGAAEHAYW	4	1.663	0.025	0.039			
	3-48	CVRDRDIVTSDSW	4	0.129	0.003				
	3-53/66	CAKENIAALGNPLDFW	4	0.975	0.001				
	3-9	CVKDMEPYGDPLRPAEAFDFW	3	4.000	0.005				
	4-34	CVRGGPINTEYWPNFDSW	4						
34012	1-18	CARTYFYGSSENRRQEYDWFDPW	5	36.47	0.003		34012	34012	34012
	3-21	CARSTRTMHQKSGMDVW	6	0.336	0.011				
	3-21	CARSTRSMHHRNSAMDVW	6	0.619	0.005				
	3-30/33rn	CARNSFYCSSISCFYRPGSKRDYYHYGM	6						
	4-30-4/31	CARGESSIONGSYVCFCDCW	4	3.713	0.016	0.137			
	4-34	CSRGAVALATHVFDYW	4	7.352	0.011				
	4-39	CARRIIIGGAFDIW	3	0.582	0.008				
	3-7	CARGTVFVLLTSYFDYW	4	0.086	0.002				
	3-7	CARGRFFFLLTSYFDYW	4	0.646	0.071				
	4-39	CARLQQWVEIW	5	0.732	0.042				
43113	4-39	CARGPVSGLTLDPW	5	10.48	0.192		43113	43113	43113
	4-39	CARRGRGWAPFDSW	4	0.366		0.257			
	4-39	CARGPVSRGDAPTPW	5	0.086	0.002				
	4-39	CATPLRDSSDYSTFDIW	3	0.151	0.033				
	4-39	CARHERDHTGFLNYYFDSW	4	0.237	0.002				
	4-39	CARRPQDFWSPYYTTYFDSW	4	7.754	0.002				
	4-39	CARLGASHYDSSGGYYYYFDYW	4	0.022		0.106			
	4-59/61	CARATRFNMHWYPFVDLW	2	1.206	0.018				
	4-b	CARPYFDGSGYYWDVLAFDVW	3	0.215	0.020				
	6-1	CARYTSGWFLDYW	4						
43213	4-59/61	CAREYSRKEGGRWRVPKTGRYSNSGM	6	0.096	0.033		43213	43213	43213
	6-1	CARRTTLGFFDYW	4						
	6-1	CARYTSGWFLDSW	5						

Table S2: PB B cell clusters connecting to the CSF/CNS compartment. See also Table 2. Each row represents a bi-compartmental cluster with contributing B cell subsets shown on the right. Columns “IgM” and IgG” show Ig-VH sequences expressed as percentage of total IgM-VH or IgG-VH reads per B cell subset or CSF. IGHV/IGHJ; closest related variable and joining germline segments utilized by Ig-VH sequences within each cluster. H-CDR3, representative H chain CDR3 aminoacid sequence per cluster, the N-terminal Cys (C) and C-terminal Trp (W) are shown for better visual orientation; SM, switched memory B cells; PC, plasma cells/plasmablasts; DN, double negative B cells. Shaded in Gray are clusters represented in the lineages shown in Figure 3 of the main manuscript.

Pat ID	14711	30512	31012	34012	43113	43213	All
SM IgG	11	1	6	6	10	1	35
PC IgG	4		1		2		7
DN IgG	1			1	1		3
SM IgM			2	1	1	2	6
DN IgM			1			1	2

Table S3: Number of bi-compartmental clusters by patient and B cell subset expressing Ig-VH related to CSF Ig-VH. Respective visual representation is shown in Figure 1 of the main article.

Pat. ID	Bi-compartmental Clusters	vs.	WBC	IgG Index	CSF Vol	CSF-IgG Counts
14711	15		103	1.5	20	20650
26712	0		1	1.3	9	4859
29612	0		4	0.5	8	5638
30512	1		7	1.2	9	18044
31012	7		1	1.2	9	7296
34012	7		4	1.4	9	14121
43113	13		3	3.1	5	4749
43213	3		10	1.1	14	6099
		r^2	0.3894	0.4561	0.1194	0.1158
		P	0.0982	0.0661	0.4019	0.4095

Table S4: Correlations between number of bi-compartmental clusters found and CSF metrics as indicated in the column title. Linear regression analysis was performed using GraphPad Prism, r^2 and P-value of each comparison are shown underneath each column shaded in gray.

Pat. ID	PB Subset	# of Clusters	# of PB Clusters connected to CSF	% of clusters connected to CSF
14711	DN.IgG	96	1	1.04
	SM.IgG	1352	11	0.81
	PC.IgG	83	4	4.82
30512	SM.IgG	100	1	1.00
31012	DN.IgM	157	1	0.64
	SM.IgM	2199	2	0.09
	SM.IgG	7269	6	0.08
	PC.IgG	643	1	0.16
34012	DN.IgG	630	1	0.16
	SM.IgM	606	1	0.17
	SM.IgG	3116	6	0.19
43113	DN.IgG	327	1	0.31
	SM.IgM	403	1	0.25
	SM.IgG	2241	10	0.45
	PC.IgG	770	2	0.26
43213	DN.IgM	111	1	0.90
	SM.IgM	531	2	0.38
	SM.IgG	481	1	0.21

Table S5: Bi-compartmental clusters in proportion to total PB B cell clusters of the indicated PB B cell subset. Only subsets with connections to CSF immune repertoires are shown.

Patient ID	Comparison N.IgM vs.	Significant?	Patient ID	Comparison N.IgM vs.	Significant?
14711	CSF.IgG	ns	31012	CSF.IgM	****
	UM.IgM	****		CSF.IgG	****
	DN.IgM	ns		UM.IgM	****
	DN.IgG	*		DN.IgM	****
	SM.IgM	ns		DN.IgG	****
	SM.IgG	ns		SM.IgM	****
	PC.IgM	***		SM.IgG	****
	PC.IgG	**		PC.IgM	****
26712	CSF.IgG	ns		PC.IgG	****
	UM.IgM	****	34012	CSF.IgM	****
	DN.IgM	ns		CSF.IgG	****
	DN.IgG	****		UM.IgM	****
	SM.IgM	****		DN.IgM	****
	SM.IgG	****		DN.IgG	****
	PC.IgM	****		SM.IgM	****
	PC.IgG	****		SM.IgG	****
29612	CSF.IgG	ns		PC.IgM	****
	UM.IgM	****		PC.IgG	ns
	DN.IgM	ns	43113	CSF.IgM	ns
	DN.IgG	*		CSF.IgG	****
	SM.IgM	ns		UM.IgM	****
	SM.IgG	ns		DN.IgM	ns
	PC.IgM	***		DN.IgG	ns
	PC.IgG	**		SM.IgM	**
30512	CSF.IgG	****		SM.IgG	****
	UM.IgM	****		PC.IgM	ns
	DN.IgM	**		PC.IgG	****
	DN.IgG	****	43213	CSF.IgM	ns
	SM.IgM	ns		CSF.IgG	ns
	SM.IgG	****		UM.IgM	***
	PC.IgM	****		DN.IgM	**
	PC.IgG	****		DN.IgG	ns
				SM.IgM	***
				SM.IgG	ns
				PC.IgM	****
				PC.IgG	****

Table S7: Comparisons of numbers of reads in each non-redundant Ig-VH sequence group between naïve PB B cells and all other B cell subsets and CSF per patient as shown in Figure 2. Each subset or CSF was compared with each other subset or CSF within a patient, resulting in 36 comparisons per patient for which CSF IgG-VH was available (14711, 26712, 19612, 30512) or 45 comparisons in patients with CSF IgG-VH and IgM-VH (31012, 34012, 43113, 43213). Naïve B cells (green type) and UM B cells frequently yielded significantly fewer numbers of reads in each non-redundant Ig-VH sequence, suggesting smaller groups of clonally related Ig-VH approximating absence of clonal expansion in these B cell subsets. These comparisons also show higher numbers of Ig-VH in groups (clusters) of clonally related Ig-VH, indirectly supporting clonal expansion in these subsets. Comparisons between subsets were made using a Kruskal-Wallis test (ANOVA with Dunn's multiple comparisons). Levels of Significance: * <0.05, ** <0.01, *** <0.001, **** <0.0001.